Environmental Pollution 182 (2013) 379–384

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Estimating the toxicity of ambient fine aerosols using freshwater rotifer *Brachionus calyciflorus* (Rotifera: Monogononta)

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A R T I C L E I N F O

Article history:
Received 29 November 2012
Received in revised form
30 April 2013
Accepted 29 July 2013

Keywords:
Freshwater rotifers
Aerosol toxicity
Hydrophobic compounds
Water-insoluble PM components

A B S T R A C T

The toxicity of atmospheric fine particulate matter ($PM_{2.5}$) in Atlanta is assessed using freshwater rotifers (*Brachionus calyciflorus*). The PM-laden quartz filters were extracted in both water and methanol. Aerosol extracts were passed through a C-18 column to separate the PM components into hydrophobic and hydrophilic fractions. Toxicity data reported in the units of LC_{50} (concentration that kills 50% of the test population in 24 h) shows that ambient particles are toxic to the rotifers with LC_{50} values ranging from 5 to 400 μg of PM. The methanol extract of the aerosols was substantially more toxic (8 ± 6 times) to the rotifers compared to the water extracts. A sizeable fraction (>70%) of toxicity was found to be associated with the hydrophobic fraction of PM. However, none of the bulk aerosol species was strongly correlated with the LC_{50} values suggesting a complicated mechanism of toxicity probably involving synergistic interactions of various PM components.

1. Introduction

Ambient particulate pollution has been linked with several adverse effects to the environment. Ambient particles have important influences on atmospheric visibility (Appel et al., 1985), climate forcing (Wang et al., 2008) and ecosystem functioning (Triolo et al., 2008). Particulate matter (PM) components such as certain metals and organic compounds [e.g. polycyclic aromatic hydrocarbons (PAHs)] can act as stressors to a variety of organisms, disrupting important ecosystem processes like nutrient cycling, grazing, and predation. The ecotoxic effects of ambient aerosols arise from the toxicity of their specific components to various populations in ecosystems.

Aquatic ecosystems are often the ultimate sinks of many environmental chemicals including atmospheric aerosols. Ambient PM end up in water bodies through both dry and wet deposition. A number of studies indicate that deposition of ambient aerosols may be an important source of contaminant loading to surface waters (Chapman et al., 2003; Golomb et al., 1997a,b). For example, significant loadings were estimated for atmospheric PAHs (5000 kg/yr) and polychlorinated biphenyls (PCBs) (1100 kg/yr) into Lake Michigan by direct measurement of their dry deposition flux [0.25–18 $\mu g/m^2$day for PAHs and 0.06–0.21 $\mu g/m^2$day for PCBs (Franz et al., 1998)]. Similar estimates (0.2–1.5 $\mu g/m^2$day) were obtained for the deposition of PAHs into Massachusetts Bay, Nahant (near Boston) (Golomb et al., 1997b). The resulting concentrations from deposition of such toxic contaminants might pose a serious threat to key populations in aquatic ecosystems. Although, human health effects of ambient aerosols are widely investigated, little is known about the ecotoxicity of atmospheric particles, particularly to aquatic environments.

Numerous assays employing different animals and microorganisms are described in the manuals published by US Environmental Protection Agency (EPA), and have been used for estimating toxicity in surface water and wastewaters (EPA, 2002). The toxicity identification evaluation (TIE) approach adopted by EPA is a stepwise procedure to characterize and identify the toxicants using a series of fractionation techniques. For example, the phase I TIE recommends the use of chronic tests with the cladoceran *Ceriodaphnia dubia* or fish *Pimephales promelas* by following an extensive sample manipulation protocol consisting of several steps such as pH adjustment, filtration, C-18 separation, and EDTA chelation (EPA, 2002). These chronic tests take several days to perform and require relatively large volumes of the test sample (~15 mL).

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http://dx.doi.org/10.1016/j.envpol.2013.07.037

Thus, although such tests are well suited to measure the overall toxicity in wastewater, the transient nature of ambient aerosol mixtures make it difficult to apply these tests for capturing the temporal variation in ambient PM toxicity.

Here we employ a fast, reliable method to measure the acute toxicity of ambient atmospheric particles to the freshwater rotifer *Brachionus calyciflorus* (Snell, 1991). Rotifers have many characteristics that favor their use as the test organisms for ecotoxicological studies: ease of culture, rapid population growth, diapausing cysts, small size, and sensitivity (Dahms et al., 2011; Snell and Janssen, 1995). Rotifers also play a key role in the dynamics of freshwater and coastal marine ecosystems and have been conventionally used for examining the toxicity of water and wastewaters (Snell, 1991, 1998). Jak et al. (1996) investigated the toxicity of a mixture of metals (As, Cd, Cr, Cu, Hg, Pb, Ni, Zn), which are in some ways similar to ambient PM samples, to various zooplankton communities of freshwater ecosystem and found rotifers to be among the most sensitive species. The importance of rotifers as primary and secondary consumers in many aquatic trophic webs is well documented (Rico-Martínez et al., 2013). In addition to these characteristics, the ability of rotifers to be cultured in very small volumes of the sample ($\sim$1 mL) makes them an ideal model for assessing the toxicity of urban atmospheric particles and performing TIEs to identify the causative agents.

We initiated rotifer 24-h acute toxicity tests to measure the relative toxicity of atmospheric aerosol components by exposing test animals to PM extracts. Ambient fine particles (Dp < 2.5 μm) collected at an urban site near central Atlanta were extracted in both water and methanol to analyze water-soluble and insoluble species. To identify the classes of compounds responsible for the observed toxicity, hydrophobic and hydrophilic fractions of the PM extracts (both water and methanol) were separated using a C-18 column and each fraction was tested. Chemical analyses of the PM samples, including organic carbon and elemental carbon (OC, EC), water soluble organic carbon (WSOC) and metals were also conducted, and the association of these aerosol properties with the toxicity responses was investigated by univariate regression analysis.

2. Experimental methods

2.1. Ambient PM sampling and chemical analyses

The details of ambient aerosol sampling for the collection of filters and their extraction in different solvents are discussed in Verma et al. (2012) and is also presented in the Supplemental information; here only a brief summary is provided. The ambient $PM_{2.5}$ were collected onto the pre-baked 8 × 10″ quartz filters (Pallflex® Tissuquartz™, Pall Life Sciences) using a high-volume sampler (Thermo Anderson, undenuded, nominal flow rate 1.13 m^3/min) at the Jefferson street site (JST); the field site used in several studies as representative of Atlanta air quality. Eight integrated samples (S1–S8; each sampled for a total volume of 1559.4 m^3 of air) and two field blanks were collected during different 23-h periods over two weeks in January–February 2012.

The particles collected on the filters were extracted via sonication in two different solvents: EPA hard synthetic freshwater [192 $NaHCO_3$, 120 $CaSO_4 \cdot 2H_2O$, 120 $MgSO_4$, 8 KCl; mg/L in all cases (referred as water from now on in the paper)] and methanol. PM concentration of the extracts used for acute toxicity tests ranged from 150 to 350 μg/mL. The methanol extracts were evaporated to near dryness using a rotary evaporator (Büchi model R-205 with B-490 heating bath; 56 °C) and then reconstituted in water.

Hydrophobic and hydrophilic components of the PM extracts were separated by passing these extracts (both water and reconstituted methanol extracts) through a C-18 (octadecyl carbon chain bonded silica; 60Å, 40–75 μm, 100 g, 20% Carbon load; Sorbent Technologies) column. The fraction that passed through the column ("hydrophilic") was collected, and the column was subsequently rinsed with methanol to partially recover the compounds retained on the column ("hydrophobic"). Methanol in the hydrophobic fraction was then evaporated and the samples were reconstituted in water.

WSOC was measured only on the water extracts of aerosols due to interference from the residual organic solvent in all other samples (i.e. methanol extracts, and both passed-through and retained fraction of PM extracts on C-18 column). Elements including metals were analyzed in both the water and methanol extracts of all PM samples ($N = 8$) and also in the hydrophilic fractions of three samples (S3, S6 and S8). Elemental and organic carbon was measured on a section (1.45 cm^2) of the collected filters. Water insoluble organic carbon (WIOC) was determined by subtracting WSOC from OC. The ambient concentration of inorganic ions during sampling was determined using an Aerosol Chemical Speciation Monitor (ACSM; Aerodyne Inc.). Since ACSM measures PM_1 instead of $PM_{2.5}$, the ions data was adjusted for the inlet size by comparing it to an online $PM_{2.5}$ PILS-IC system [(Particle-Into-Liquid-Sampler coupled to an ion chromatograph) (Weber et al., 2001)] that ran concurrently at the site for a fraction of the filter sampling period (February 01–10, 2012). $PM_{2.5}$ mass was measured continuously by a tapered element oscillating microbalance (Thermo Scientific TEOM 1400a). Chemical composition of the ambient particles for the various filter sampling periods is shown in Supplemental information and is also discussed in a previous publication (Verma et al., 2012).

2.2. 24-h acute toxicity test with Brachionus calyciflorus

A 24-h acute toxicity test using the rotifer *B. calyciflorus* Pallas 1766 hatched from resting eggs was conducted on both the water and reconstituted methanol extracts of PM, and also on the respective C-18 column pass-through and retained fractions (Snell et al., 1991). We used the modified TIE protocol by adjusting it to the conditions for ambient PM samples (EPA, 2002). For example, instead of using *Ceriodaphnia dubia* and/or *Pimephales promelas* chronic toxicity tests, we used a 24-h acute toxicity test with *B. calyciflorus*. This change is important for various reasons: a) it allows analysis of small (1 mL) volumes, b) it allows for the rapid and multiple tests to be performed easily, c) being an acute test, it allows assessment of the high levels of toxicity expected from the aerosol samples. The procedural details of the test are described in Snell et al. (1991) and the standardized ASTM protocol (Snell, 1991). Briefly, dry cysts of *B. calyciflorus* were hatched by hydrating in EPA medium at 25 °C and continuous light (4000 lux of cool white fluorescent illumination) for 24 h. The conditions for the toxicity tests were as follows: 25 °C, pH 7.4–7.8, hardness 80–100 mg $CaCO_3$/L, dim light. The tests were conducted in 24-well polystyrene plates (Costar Co, USA). Ten neonate females were placed in each well filled with a test volume of 1 mL. The test required no feeding or renewal of the medium. We performed a series of dilutions (100, 50, 25, 12.5, 6.25 and 3.13%) for each sample. Fifty test animals (10 per well, five replicates, in five independent experiments) were used for each blank and sample dilution. Mortality was scored after 24 h as rotifer immobility and the data was analyzed by means of the software Statistica 7 (Statsoft Inc., 2002) to determine LC_{50} from linear regression. Tests were considered valid only if mortality was less than 10% in the controls.

3. Results

3.1. Comparison of water and methanol extract LC_{50}

Fig. 1 shows the results of rotifer acute toxicity tests conducted on the water and methanol extracts of PM samples (identified as

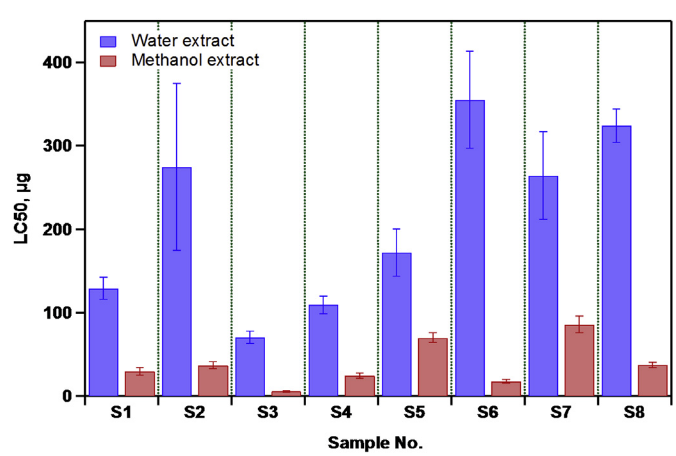

Fig. 1. Results of the rotifer acute toxicity test conducted on the water and methanol extracts of various $PM_{2.5}$ samples. Note: 1. Samples S1–S8 represent different filters collected from JST site on different days in January–February 2012. For details on the filter sampling such as schedule, duration and mass loadings, refer Verma et al. (2012). 2. Error bars denote the 95% confidence intervals.

samples 1 through 8, S1–S8). Toxicity is expressed in units of PM mass (μg) that causes 50% mortality of the test population [(LC_{50}, lethal concentration)]; lower LC_{50} implies more toxic samples. The results expressed in these units are independent of the ambient PM concentrations and thus allow the comparison of relative toxicity in different filter samples. As shown, the toxicity of the methanol extracts of PM samples is much higher than the water extracts - average LC_{50} of methanol extracts is 8 ± 6 times lower than the water extracts. Methanol-extracted $PM_{2.5}$ components would include most of the water-insoluble compounds with some fraction of water-soluble species. Thus, these results indicate that non-polar compounds are more toxic to the rotifers compared to polar compounds.

3.2. Removal of toxicity by the C-18 column

Table 1 shows the results of rotifer acute toxicity tests after passing the PM extracts (both water and methanol) through the C-18 column (i.e. hydrophilic extract). In this case, the toxicity response is expressed in terms of the mean mortality of rotifers since the samples with low mortality (less than 50%) have high uncertainty in the calculated LC_{50} values. Note that the results presented in these units (% mortality) are a function of extract (ambient) concentrations and limit the direct comparison of toxicity removal among different samples. For example, the percentage removal of toxicity for two samples having similar mortality of their hydrophilic fractions might be very different depending upon the LC_{50} values of their original extracts. Thus, an exact quantification of the percentage removal of toxicity by C-18 column is not possible from these results; however, they allow two important observations: 1) The hydrophobic fraction dominates over hydrophilic fraction in the toxicity of ambient PM such that more than approximately 70% toxicity [original mean mortality (100%) − averaged mean mortality after C-18 (25% for water and 31% for methanol extracts)] is removed by the C-18 column. 2) The contribution of hydrophobic matter to toxicity is higher in methanol extracts than water extracts given the much lower LC_{50} for the former. Similar results were obtained for the oxidative potential of these samples measured by dithiothreitol (DTT) assay, i.e. higher DTT activity was observed for the methanol extracts compared to water extracts, most of which was associated with hydrophobic compounds (Verma et al., 2012). Although a direct correspondence of the DTT assay and the acute toxicity test using rotifers cannot be established merely based on these results and without the details of mechanistic pathways of toxicity in rotifers, it is possible that certain common components, which might be concentrated in the hydrophobic fraction, at least are partially responsible for the response of both assays.

Table 1
Mean mortality of the rotifers measured in the acute toxicity test for the passed-through (hydrophilic) fractions of PM extracts (both water and methanol).

Sample no.	Mean ($\pm 1\sigma$) mortality (%) for passed-through (hydrophilic) fraction	
	Water extract	Methanol extract
S1	10 ± 10	35 ± 10
S2	0 ± 0	0 ± 0
S3	28 ± 5	28 ± 13
S4	3 ± 5	10 ± 10
S5	23 ± 17	20 ± 16
S6	100 ± 58	100 ± 0
S7	15 ± 13	57 ± 40
S8	20 ± 16	3 ± 5

3.3. Correlation of rotifer toxicity with PM components

Table 2 shows the results of univariate linear regression (R and p values) between the measured chemical components and LC_{50} values for both water and methanol extracts of PM. Based on the approach followed in similar studies investigating the relationship between chemical species and toxicity response, all of the regression was conducted on mass normalized levels of toxicity and PM components. The most correlated species with toxicity in the water extracts of aerosols are EC, WIOC, and OC. Certain metals such as As and Cd are also correlated with LC_{50} values of the water extracts. Inorganic ions which constitute a significant mass of the ambient PM are not correlated with toxicity response of either water or methanol extracts of PM. None of the measured chemical species is correlated with LC_{50} of the methanol extracts of PM, despite it being more intrinsically toxic than water-soluble components.

3.4. Partitioning of PM metals into the hydrophobic and hydrophilic fractions

Fig. 2 shows the results of partitioning of PM elements (including metals) on the C-18 column for both water and methanol extracts, in terms of their percentage components in the hydrophobic fraction (average of three samples S3, S6 and S8). The hydrophobic percentage was calculated by subtracting the percentage of hydrophilic metals from 100%. The concentration (μg/L) of metals in both water and methanol extracts of these samples (S3, S6 and S8) and their respective hydrophilic fractions used for the toxicity tests is provided in Supplemental information (Table S5). To test if the removal of these metals by the C-18 column is significant, we conducted a one sample t-test on the concentration of metals in initial extracts (containing both hydrophilic and hydrophobic components) and their respective concentration in the hydrophilic fractions. The results of t-test (not shown here) revealed that certain metals such as V, Mn, Zn, Cd and Fe were significantly

Table 2
Results of a univariate regression analysis between PM constituents and LC_{50} values of ambient PM samples measured in the rotifer acute toxicity test ($N = 8$).

PM component	Correlation coefficient and significance level			
	Water extract		Methanol extract	
	R	p	R	p
OC	**−0.68**	0.06	−0.04	0.93
EC	**−0.78**	0.02	−0.28	0.50
SO_4^{-2}	0.70	0.05	0.03	0.95
NO_3^-	0.28	0.51	0.05	0.91
NH_4^+	0.68	0.06	0.02	0.96
WSOC	−0.29	0.49	0.05	0.90
WIOC	**−0.69**	0.06	−0.07	0.87
Al	−0.14	0.74	0.03	0.94
S	0.76	0.03	0.06	0.89
K	−0.22	0.60	−0.27	0.52
Ca	0.54	0.17	−0.44	0.28
V	0.23	0.59	0.16	0.71
Mn	−0.34	0.41	−0.30	0.48
Fe	0.18	0.68	0.39	0.34
Co	−0.14	0.75	−0.42	0.30
Ni	−0.05	0.90	−0.39	0.34
Cu	0.21	0.62	−0.42	0.31
Zn	0.21	0.62	0.10	0.82
As	**−0.73**	0.04	−0.52	0.19
Cd	**−0.68**	0.06	−0.51	0.19
Ba	−0.24	0.57	−0.38	0.35
Pb	−0.40	0.33	−0.23	0.58

Regression was done on the mass normalized levels of both rotifer toxicity and PM constituents.
Bold values represent $R < -0.60$.

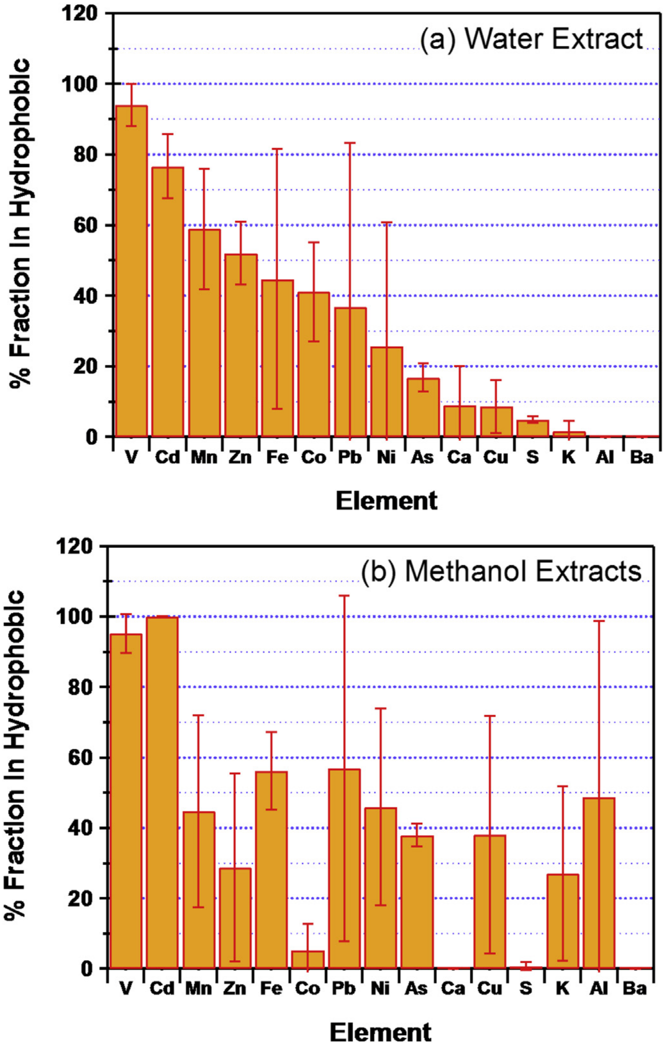

Fig. 2. Percentage of PM metals in the hydrophobic fraction of both water (a) and methanol (b) extracts of PM samples (average of three samples S3, S6, S8; error bars are one standard deviation). Note: Hydrophobic percentage was calculated by subtracting the percentage in hydrophilic fraction from 100%.

($p < 0.05$) removed on the column while other elements (Al, S, K, Ca, Co, As, Ni, Ba, Pb and Cu) were less affected and partitioned mostly in the hydrophilic fraction.

4. Discussion

Our study demonstrates the importance of water-insoluble PM species in contributing to the toxicological properties of ambient aerosols. We note that the exposure mode of these aerosols to rotifers in our tests are not exactly representative of the natural environment where the interaction of PM species with organisms predominately occurs through the particles' surface (Nygaard et al., 2004). Methanol extraction likely solubilizes the species from deeper inside the particles, which would otherwise not be immediately bioavailable. However, these water-insoluble species might gradually get exposed to the aquatic animals over a prolonged period of time and eventually become bioavailable. For example, particles could be deposited in water bodies and be ingested by rotifers that naturally graze on microalgae in the 1–10 μm range. Little is known about the bioavailability of PM toxicants as they pass through the digestive system of animals. Thus, methanol based extraction of PM filters is likely biologically relevant and can be considered as a tool to accelerate the exposure conditions for such water-insoluble PM species.

The regression analysis alone on the measured species and LC_{50} values of the PM samples didn't yield any conclusive association of rotifer toxicity with PM chemical composition. For example EC and WIOC, which are correlated with toxicity in the water extracts of aerosols are generally used as the markers of primary aerosols emissions [e.g. vehicular (Schauer, 2003)], and are not present in the water extracts. Thus their correlation with LC_{50} implies a statistical artifact driven by the collinearity of these primary species with other unidentified toxic components in the water extracts of PM. Other correlated metals (As and Cd) are also emitted from the primary vehicular sources (Popescu, 2011; Talebi and Abedi, 2005) and are correlated with EC ($R = 0.87$ for As and 0.53 for Cd; Table S2 in supplemental information). The collinearity of these species suggests that the mechanism of toxicity in rotifers is more complicated than what can be inferred from a pure statistical correlation. Previous studies on estimating aerosol toxicity using other methods such as Microtox® (Lin and Chao, 2002; Oanh et al., 2002; Turóczi et al., 2012) and MitoScan™ (Doherty and Gustavson, 2002; Sheesley et al., 2004) likewise have not reported any association with PM chemical composition. In comparison to these biological tests, the chemical assays measuring the oxidative properties of PM have been well documented to be driven by the specific PM compounds (Cho et al., 2005; DiStefano et al., 2009). For example, in our previous study on the same set of samples, the response of the acellular DTT assay was found to be well correlated with organic compounds (Verma et al., 2012). Such disparity in deriving the association between assays' response from PM chemical composition indicates the complexity of extrapolating the results of chemical assays to a whole animal toxicity test. In a multicellular animal like the rotifer, the combined contribution of different PM constituents and their synergistic effects might play a major role in causing mortality, unlike the chemical assays which simulate only a few of the many potential pathways of PM toxicity.

Despite these complexities, the physical separation of PM components on the C-18 column helps to roughly identify the bulk group of species responsible for rotifer toxicity. Perhaps the most interesting observation in our experiments is that the compounds which appear to be highly toxic to rotifers are mainly hydrophobic. Conventionally, the hydrophobic organic aerosol is termed as HULIS (Humic-like-substances) because of its apparent similarity to the terrestrial/aquatic humic and fulvic acids (Graber and Rudich, 2006). Although, aquatic humic substances are predominantly formed naturally from the biodegradation of terrestrial plants, there have been studies documenting the adverse effects of these compounds on various aquatic animals. Timofeyev et al. (2006a; 2006b) isolated the natural organic matter (NOM; consisting mainly of humic substances) from the brown-water lake in Brandenburg State (Germany) and examined its deleterious effects on the freshwater amphipods [*Gammarus lacustris* Sars, *Gammarus tigrinus* (Sexton), *Eulimnogammarus verrucosus* (Gerstf.) and *Eulimnogammarus cyaneus* (Dyb.)]. The principal mode of toxicity was found to be associated with the oxidative stress initiated by ROS (reactive oxygen species) generation. Although, a precise similarity between the compounds in atmospheric HULIS and aquatic humic substances is not established, these results support our finding that the compounds mostly responsible for the rotifer toxicity have a common chemical signature characterized by strong hydrophobicity.

Since our regression results tend to indicate that metals (e.g. As and Cd) might also contribute to rotifer toxicity, we investigated their partitioning behavior in the different fractions (i.e. hydrophobic and hydrophilic) separated by the C-18 column. There is limited literature available on the partitioning of metals in solid phase extraction (SPE) columns. Lin and Yu (2011) passed the aerosol extracts collected from Pearl River Delta region (China) through an Oasis HLB (Waters) column and found that most of these elements were hydrophilic. Our results (Fig. 2) are largely in agreement with their observations, with a few exceptions, e.g., V, Mn, Zn, Cd, and Fe. The removal of some metals (V, Mn, Zn, Cd, and Fe) on the C-18 column indicates that these are probably complexed with hydrophobic organic compounds. Collectively, these data may help to determine if any causal relationship exists which could explain the observed correlations of certain metals with LC_{50}. For example, the predominant partitioning of As in the hydrophilic fraction of PM does not mechanistically support its statistical correlation with LC_{50} of the PM extracts. In contrast, the plausible complexation of metals with organic compounds (Chapman et al., 2003) partitioned in the hydrophobic fraction would likely render them inactive, which might otherwise be toxic to rotifers. Thus, overall these results do not provide enough evidence to show that the ambient PM metals measured in our study have any major contribution to rotifer toxicity. For an accurate assessment of the metals role in rotifer toxicity and the associated ecological risk, further information [e.g. metal speciation, bioavailability, and uptake route as outlined in Chapman et al. (2003)] would be required.

Various researchers have examined the partitioning behavior of organic compounds on the SPE column and their studies consistently indicate chemically different classes of compounds in the hydrophobic and hydrophilic fractions. For example, aromatic hydrocarbons have been found to be exclusively associated with hydrophobic components while the hydrophilic fraction consists of mostly aliphatic substances (Sannigrahi et al., 2006). Thus, one class of compounds that remains as a viable candidate responsible for rotifer toxicity is the aromatic hydrocarbons, including PAHs and quinones. These aromatic hydrocarbons are found to be highly redox-active in a number of studies (Li et al., 2012; Shang et al., 2012). The higher solubility of these aromatics in organic solvents (e.g. methanol) than water is also consistent with the elevated toxicity response (lower LC_{50}) of the methanol extracts of PM than water extracts (Duffy, 2012).

To investigate if certain aromatic hydrocarbons are responsible for at least a part of the rotifer toxicity in our tests, we evaluated toxicity of two model compounds −1,4 Naphthaquinone and 9,10 Phenanthroquinone. Both quinones were found to be highly toxic to the rotifers with LC_{50} values of 20.40 μg/L (of extracts) for Naphthaquinone and 33.17 μg/L for Phenanthroquinone. These values are of the same order of magnitude as the concentration of these species in our test PM extracts [5–15 μg/L for Naphthaquinone and 10–30 μg/L for Phenanthroquinone, as estimated from their typically reported ambient concentrations (Cho et al., 2004)]. Although, pure compounds tested in our experiment neither represent the chemical state (e.g. complexed or free) of these species in ambient PM, nor account for any synergistic relationships with other PM components, they at least provide some hints on the potential role of certain aromatic hydrocarbons (e.g. quinones and/or quinone-like structures) in rotifer toxicity.

Our tests were conducted at a relatively high exposure concentrations (150–350 μg of PM/mL) in order to have a comparative measure of toxicity of different PM samples (i.e. determining the LC_{50} values). The concentration of various toxicants in an aquatic environment and their fraction attributable to the ambient PM pollution depends upon a number of factors such as ambient aerosols concentration, atmospheric mixing height, volume of the water body, catchment area, and the rate of dilution of receiving waters. The high spatial and temporal variability associated with each of these factors make it beyond our scope to assess the relevance of concentrations tested in our experiments to the natural environment. Further, the ultimate fate of a chemical in aquatic ecosystems is a complex function of various biophysicochemical processes such as its persistence, competitive uptake by other sinks and bioaccumulation [as discussed in Alvarado-Flores et al. (2012)]. All these considerations challenge the translation of our results to an accurate assessment of the overall toxicity burden of ambient particles on aquatic environments in general.

Extrapolation of the results of our study to natural environment is also limited by the size range of the PM sampled. Although $PM_{2.5}$ forms a major fraction of the respirable suspended PM which is most associated with the observed human health effects (Ballester et al., 2006), the larger particles [e.g. coarse PM (PM_{10}–$PM_{2.5}$)] would also have a substantial deposition flux and thus might be important in the context of present study. Furthermore, the small sample size ($N = 8$) makes it imperative to use caution while interpreting our results. The limited statistical power with such a small sample size probably contributes to the lack of a consistent correlation of rotifer toxicity with PM components. We also note that none of our tests, whether statistical (i.e. correlation of LC_{50} with PM components) or mechanistic (i.e. separation of PM components into hydrophobic and hydrophilic fractions, and testing quinones for their individual toxicity) provide strong evidence to confirm a specific chemical component driving the entire toxic response of PM samples in rotifers. An improved understanding of the ecological impacts of ambient PM and their toxicity to natural populations might be obtained by conducting such studies on a larger sample size over an extended time scale.

Acknowledgments

This publication was made possible by USEPA grant R834799. Its contents are solely the responsibility of the grantee and do not necessarily represent the official views of the USEPA. Further, USEPA does not endorse the purchase of any commercial products or services mentioned in the publication. The authors thank the Mexico-US Commission for Cultural and Educational Exchange and to the Fullbright program for providing the Fulbright/García-Robles AWARD to Roberto Rico-Martinez that supported his stay at the Georgia Tech, and the Air Resources Engineering Center at Georgia Tech for partial support of Vishal Verma. Cody Zipperer contributed to the execution of the rotifer toxicity tests. We also appreciate the contribution of Martin Shafer at University of Wisconsin, Madison for conducting the metals analyses on PM extracts. We thank Eric Edgerton and the SEARCH network for use of the JST site.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2013.07.037.

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